Sudan University of Science and Technology

Collage of Graduate Studies

Determination of Prothrombin Time, Activated Partial Thromboplastin Time and plasma fibrinogen level among Hypertensive, Diabetic and Normal pregnant women at third trimester in Khartoum State

تحديد زمن الثرومبين وزمن الثرومبوبلاستين الجزئي المنشط و مستوي الفبرينوجين البلازمي لدى الحوامل المصابات بارتفاع ضغط الدم ومرض السكري والحوامل الصحيحات في الثلث الأخير من الحمل في ولاية الخرطوم

A dissertation Submitted for partial fulfillment for the Requirements of M.Sc degree in Medical Laboratory Science-Hematology and Immunohematology

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قال تعالي:

(آمَنَ الرَّسُولُ بِمَا أُنْزِلَ إِلَيْهِ مِنْ رَبِّهِ وَالْمُؤْمِنُونَ كُلٌّ آمَنَ بِاللَّهِ وَمَلائِكَتِهِ وَكُتُبِهِ وَرُسُلِهِ لا نُفَرِّقُ بَيْنَ أَحَدٍ مِنْ رُسُلِهِ وَقَالُوا سَمِعْنَا وَأَطَعْنَا غُفْرَانَكَ رَبَّنَا وَإِلَيْكَ الْمَصِيرُ(285) لا يُكَلِّفُ اللَّهُ نَفْساً إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلا تَحْمِلْ عَلَيْنَا إِصْراً كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلا تُحَمِّلْنَا مَا لا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ (286))

صدق الله العظيم

سوره البقرة ـ الآيات(285- 286)

Dedication

I dedicate this work to… my parents who mean the world to me

To the best gift given to me from Almighty Allah, To the soul of my father who had always been source of encouragement

To my sweet mother who has been always there to support me and make my life shining

And to everyone who smile on my face and help me.

ACKNOWLEDGMENT

Abstract

This was analytical case –control study conducted in Khartoum State during the period from September to December 2015. The aim of this study was to compare the prothrombin time, activated partial Thromboplastin time and plasma fibrinogen level between hypertensive pregnant, diabetic pregnant, normal pregnant and control group. A total of 120 consecutive females were enrolled;30 pregnant with hypertension, 30 pregnant with diabetes, 30 normal pregnant and 30 from healthy females (control group). 2.7 ml of blood samples were collected in 3.2% tri-sodium citrate after informed consent taken from each female . Coagulation profile was estimated using Dia med coagulometer. The mean of age in pregnant with hypertension was (28.97 ± 6.14 years), pregnant with diabetes was (31.03 ± 5.55 years),normal pregnant was(26.97 ± 6.80 years) and control group(28.03 ± 7.44 years).PT was significantly decreased in hypertensive pregnant (13.82 ± 2.10 sec) and diabetic pregnant(12.61 ± 1.37 sec) compared to control group(15.48 ± 2.65sec)(*p.value* 0.010) , (*p.value* 0.000)respectively, and insignificantly difference between normal pregnant (15.01 ± 1.94 sec) and control group (*p.value* 0.434) and significantly decreased in pregnant with hypertension and pregnant with diabetes compared to normal pregnant (*p.value* 0.029) (*p.value* 0.000) respectively, significantly decrease in diabetic pregnant compared to hypertensive pregnant(*p.value* 0.026).APTT was significantly decreased in hypertensive pregnant(34.28 ± 3.48 sec) and diabetic pregnant (35.60 ± 4.83 sec) compared to control group(40.39 ± 4.79sec)(*p.value* 0.000 for both) and insignificantly difference between normal pregnant(40.88 ± 3.82 sec) and control group (*p.value* 0.491) and significantly decreased in pregnant with hypertension and pregnant with diabetes compared to normal pregnant (*p.value* 0.000 for both) and insignificant difference in diabetic pregnant compared to hypertensive pregnant(*p.value* 0.236).fibrinogen level was significantly increased in

hypertensive pregnant(520.23±68.05 mg/dl), diabetic pregnant(548.83±99.71 mg/dl), normal pregnant(398.23±38.39 mg/dl) compared to control group(268.37±59.67 mg/dl) (*p.value* 0.000 for all) and insignificant difference between hypertensive pregnant and diabetic pregnant(*p.value* 0.116) and significant increase in hypertensive pregnant and diabetic pregnant compared to normal pregnant (*p.value* 0.000 for both). This results analyzed by the program of Statistical Package for Social Science version 16. In conclusion fibrinogen was significantly increased in hypertensive pregnant, diabetic pregnant and normal pregnant so measurement of PT , APTT and plasma fibrinogen level may be predictor of thrombosis in pregnancy.

المستخلص

هذه دراسة حاله مقارنه أجريت في ولاية الخرطوم في الفترة ما بين سبتمبر إلى ديسمبر 2015. وقد هدفت لمقارنه زمن الثرومبين ،زمن الثرومبوبلاستين الجزئي المنشط و مستوي الفبرينوجين بين الحوامل المصابات بارتفاع ضغط الدم ، الحوامل المصابات بالسكري والحوامل الأصحاء وعينه ضابطه. شملت هذه الدراسة 120 سيده. 30 منهم حوامل مصابات بارتفاع ضغط الدم ، 30 حوامل مصابات بالسكري، 30 حوامل أصحاء و30 فتاه كعينه ضابطه. جمعت 2.7 مل من الدم في 3.2% ثلاثي سترات الصوديوم بعد اخذ الموافقة المستنيرة من المشاركات . قيست اختبارات التجلط بواسطة جهاز الكواقلوميتر(بايوميد). كان متوسط أعمار مرضى حوامل ارتفاع ضغط الدم (28.97±6.14 سنه ، حوامل مرضي السكري (31.03±5.55 سنه) الحوامل الأصحاء (26.97±6.80 سنه) والعينة الضابطة (28.03±7.44 سنه). أظهر التحليل الإحصائي انخفاضا في زمن الثرومبين لدى الحوامل مرضى ارتفاع ضغط الدم(13.82±2.10 ثانيه) والحوامل مرضى السكري (12.61±1.37 ثانيه) عند مقارنته بالعينة الضابطة (15.48±2.65 ثانيه) القيمة المعنوية (0.010) (0.000) بالترتيب ، بينما لا يوجد اختلاف ذو دلاله وصفيه بين الحوامل الأصحاء (15.01±1.94) والعينة الضابطة ،القيمة المعنوية (0.434).اظهر التحليل الإحصائي نقصانا في زمن الثرومبين لدي الحوامل مرضي السكري وضغط الدم عند مقارنته بالحوامل الأصحاء القيمة المعنوية (0.029)(0.000) بالترتيب ونقصان ذو قيمه معنوية بين الحوامل مرضى السكري عند مقارنتهم بمرضى ضغط الدم القيمة المعنوية(0.026). أظهر التحليل الإحصائي انخفاضا في زمن الثرومبوبلاستين الجزئي المنشط لدى الحوامل مرضى ضغط الدم(34.28±3.48 ثانيه) والحوامل مرضى السكري(35.60±4.83 ثانيه) عند مقارنتهم بالعينة الضابطة(40.39±4.79 ثانيه)القيمة المعنوية (0.000 للاثنين معا) ،بينما لا يوجد فرق ذو دلاله وصفيه بين الحوامل الأصحاء (40.88±3.82 ثانيه) والعينة الضابطة، القيمة المعنوية (0.491) ووجد أن هناك انخفاض ذو دلاله وصفيه عند الحوامل مرضى ضغط الدم ومرضى السكري عند مقارنتهم مع الحوامل الأصحاء ، القيمة المعنوية (0.000 للاثنين معا) ، بينما لا يوجد فرق ذو دلاله وصفيه بين الحوامل مرضى السكري عند مقارنتهم بمرضى ضغط الدم، القيمة المعنوية (0.236). أظهر التحليل الإحصائي ارتفاعا ذو دلاله وصفيه في مستوى الفبرينوجين عند الحوامل مرضى ضغط الدم(520.23±68.05 مج\ديسي)، الحوامل مرضى السكري(548.83±99.71 مج\ديسي)، والحوامل الأصحاء (398.23±38.39 مج\ديسي) عند مقارنتهم بالعينة الضابطة (268.37±59.67 مج\ديسي)، القيمة المعنوية (0.000 للكل) ، بينما لا يوجد فرق ذو دلاله وصفيه بين الحوامل مرضى السكري ومرضى ضغط الدم ، القيمة المعنوية (0.116)، وأيضا وجد أن هناك ارتفاع ذو دلاله وصفيه عند الحوامل مرضى ضغط الدم ومرضى السكري عند مقارنتهم بالحوامل الأصحاء، القيمة المعنوية (0.000

للاثنين). حللت النتائج بواسطة برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة 16. خلصت الدراسة إلى زيادة مستوى الفبرينوجين لدى الحوامل مرضى ارتفاع ضغط الدم، مرضى السكري والحوامل الأصحاء، لذا قياس مستوى الفبرينوجين ، وزمن الثرومبين وزمن الثرومبوبلاستين الجزئي المنشط قد يكون مؤشر في الكشف عن تجلط الدم عند الحوامل.

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Abbreviation

ADP   Adenosine diphosphate

APC   Activated Protein c

APTT   Activated Partial Thromboplastin Time

BP   Blood pressure

DIC   Disseminated Intravascular Coagulation

DM   Diabetes Mellitus

FDP   Fibrinogen Degradation Product

GP   Glycoprotein

GP V   Glycoprotein 5

HCG   Human Chorionic Gonadotropin

HMWK   High Molecular Weight Kininogen

HPL   Human Placental Lactogen

HRG   Histidine Rich Glycoprotein

HTN   Hypertension

IDDM   Insulin Dependent Diabetes Mellitus

Ig G   Immunoglobulin G.

KD   kilo Dalton

LNMP   Last Normal Menstrual Period

MPV   Mean Platelet Volume

PAI-1   Plasminogen Activator Inhibitor -1

PE   Preeclampsia

Phl   phospholipid

PT   Prothrombin Time

RNA   Ribonucleic Acid

T2D   Type 2 Diabetes

TA2   Thromboxane A2

TAFI   Thrombin Activated Fibrinolysis Inhibitor

TF   Tissue Factor

TFPI   Tissue Factor Pathway Inhibitor

t-PA   tissue Plasminogen Activator

TT   Thrombin Time

u-PA   urokinase Plasminogen Activator

VEGF   Vascular Endothelial Growth Factor

vWF   von Willebrand Factor

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction and literature Review

1.1 Introduction:

Hemostasis is one of the most significant maintenance system of human body , and maintain the liquid state of circulatory blood and prevent bleeding that result from blood vessels damage. Bleeding after injury is stopped in three stages: vascular stage, platelets stage and blood coagulation stage (Baklaja *et al.*, 2008).

Fibrinogen, also called Factor I, it is a blood plasma protein produced by the liver that plays an important role in blood coagulation. Blood coagulation is a process in which several components of the blood forma clot. When blood escapes from a rupture in a blood vessel, coagulation is triggered. Several proteins, called coagulation factors, go into action to produce thrombin. The thrombin then converts fibrinogen to fibrin. Fibrin produced from fibrinogen is the main protein in a blood clot (Lupien *et al.*,2004).

Pregnancy is a unique period in a woman's lifetime. A number of anatomic, physiologic, biochemical and psychological changes take place. These changes may easily be misinterpreted by physicians who lack experience in regards to pregnancy effects on a woman's body. It is important that physicians caring for women understand the implications of these physiological changes in order to avoid any diagnostic errors and errors of management (Kofins, 2015).

Hypertensive disorders complicating pregnancy are common and form one of the deadly triad along with hemorrhage and infection that results in maternal and perinatal morbidity and mortality (Naaz *et al.,*2015).

Also Pregnancy is associated with changes in insulin sensitivity which may lead to changes in plasma glucose levels. For women with known diabetes or for women who develop diabetes during the pregnancy, these changes can put outcomes at risk. In study done by Home and colleges 2009 to identify women for whom such problems are new, and helping them, as well as women already

known to have diabetes, so as to achieve the desired outcome of a healthy mother and baby (Home *et al*., 2009).

1.2 Literature review:

1.2.1 Hemostasis:

The processes of blood coagulation and fibrinolysis are the primary defense systems of the vasculature. The opposing forces of fibrin clot formation and dissolution maintain hemostasis and preserve vascular function and integrity. Procoagulant events that culminate in a thrombin generation and fibrin clot formation protect the vasculature from perforating injury and excessive blood loss. Fibrinolysis removes the fibrin clot, restores blood flow, and initiates mechanisms involved in tissue repair and regeneration. Hemostasis, therefore, refers to multiple discreet processes that center on a-thrombin generation, fibrin clot formation, and fibrin clot dissolution (Greer *et al*., 2003).

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and their inhibitors. When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding and essential for normal hemostasis: (1) vessel wall contraction, (2) platelet adhesion and aggregation (platelet plug formation), and (3) plasmatic coagulation to form a fibrin clot. All three mechanisms are essential for normal hemostasis (Munker *et al*.,2007).

When a blood vessel becomes damaged, as a result of surgery or by a catheter, or some other means, there is some degree of local vasoconstriction. However the primary event is the adhesion of circulating platelets to the damaged vessel wall and simultaneous activation of the classical coagulation cascade, resulting in activation of thrombin and leading to the conversion of fibrinogen into fibrin. A primary haemostatic plug is produced, followed by fibrinolytic activity and hopefully repair of the damaged vessel wall. To prevent inappropriate activation of these different pathways there is now a series of very well characterized inhibitory pathways (Galley, 2002).

1.2.1.1 Vascular system:

The vascular system prevents bleeding through vessel contraction, diversion of blood flow from damaged vessels, initiation of contact activation of platelets with aggregation, and contact activation of the coagulation system. The vessel wall contains varying amounts of fibrous tissue such as collagen and elastin, as well as smooth muscle cells and fibroblasts. Arteries are the vessels that take blood away from the heart and have the thickest walls of the vascular system. Veins return blood to the heart, and are larger with a more irregular lumen than the arteries. Veins, however, are thin walled, with elastic fibers found only in larger veins. Arterioles are a smaller subdivision of arteries, and venules are smaller subdivisions of veins. Capillaries are the thinnest walled and most numerous of the blood vessels. They are composed of only one cell layer of endothelium that permits a rapid rate of transport materials between blood and tissue (Ciesla, 2007).

1.2.1.2 The Endothelium:

The endothelium actively affects the function of all Hemostasis components. The endothelium has two roles: activation and inhibition of Hemostasis (Baklaja *et al*., 2008).

The endothelium contains connective tissue such as collagen and elastin. This matrix regulates the permeability of the inner vessel wall and provides the principal stimuli to thrombosis following injury to a blood vessel. Circulating platelets recognize and bind to insoluble subendothelial connective tissue molecules. This process is dependent on molecules that are in plasma and on platelets. Two factors, von Willebrand (vWF) and fibrinogen, participate in the formation of the platelet plug and the insoluble protein clot, resulting in the activation of the coagulation proteins. Receptor molecules adhere to platelets and damaged vessel components, also allow platelets to use vWF and fibrinogen to bind platelets and form a plug. Blood flows out through the wall and comes in contact with collagen (Ciesla, 2007). Collagen is an insoluble fibrous protein

that accounts for much of the body's connective tissue. Vessel injury leads to the stimulation of platelets. Platelets contain more of the contractile protein actomyosin than any cells, other than muscle cells, giving them the ability to contract. Basically platelets adhere to collagen and other platelets adhere to them. A plug is built and the platelets' ability to further contract compacts the mass. In forming the initial plug, platelets have now built a template on a lipoprotein surface, which in turn activates tissue factor. The balance between coagulation proteins and anticoagulants now leans toward coagulation. This process will accelerate vasoconstriction, platelet plug development, and the formation of crosslinked fibrin clot (Ciesla, 2007).

1.2.1.3 Platelets:

Platelets are produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoetic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin. Once released from the bone marrow young platelets are trapped in the spleen for up to 36 hours before entering the circulation, where they have a primary haemostatic role. Their normal lifespan is 7-10 days and the normal platelet count for all age groups is $150\text{-}450\text{x}10^{9}$/l. The mean platelet diameter is 1-2µm and the normal range for cell volume (MPV) is 8-11 fl. Although platelets are non-nucleated cells, those that have recently been released from the bone marrow contain ribonucleic acid (RNA) and are known as reticulated platelets. They normally represent 8-16% of the total count and they indirectly indicate the state of marrow production (Provan, 2003).

Normal physiological response to vascular injury includes rapid platelet adhesion on the subendothelium and generation of thrombin, which induces platelet aggregation and fibrin formation (Baklaja *et al*., 2008).

1.2.1.3.1 Platelet adhesion:

following blood vessel injury, platelets adhere to the exposed subendothelial matrix via specific glycoprotein (GP). Under condition of high shear, e.g. arterioles, the exposed subendothelial matrix is initially coated with VWF multimeres.

The platelets than make contact with VWF via the GPIb-XI-V complex on platelets. This itutiates platelet rolling in the direction of blood flow over the ex posed VWF with activation of GPIIb/IIIa receptors. Firm adhesion is established by the slower but stronger interaction of other glycoproteins including activated GPIIb/IIIa with VWF and GPVI and integrin α1/ β2 with collagen and other component of the subendothelial matrix. Under static or low shear conditions, platelets adhere predominantly to collagen of the subendothelium. Collagen bind initially to GPIa/IIa, cross-links many of these integrin molecules, and in this way activates platelets(Hoffbrand A.V, *et al*., 2006).

1.2.1.3.2 Platelet activation:

During adherence and activation, platelets change from discs into spheres with projections on long pseudopods. Platelet plug is developed further on the initial layer of adherent platelets by aggregation of platelets recruited to the site of vessel injury (Baklaja *et al*., 2008).

1.2.1.3.3 Platelet release reaction:

Platelets undergo aggregation and release the contents of their dense granules and α-granules when exposed to agonists such as adenosine diphosphate (ADP), epinephrine, thrombin or collagen (Baklaja *et al*., 2008).

Dense granules release calcium, serotonin and ADP, the latter of which promotes continued aggregation. The secreted α-granule content includes platelet factor 3,β-thromboglobulin,platelet-derived growth factor, thrombospondin, factor V and plasma proteins such as fibrinogen and immunoglobulinG(IgG). ADP and epinephrine, otherwise weak agonists for

platelet aggregation, require prostaglandin, thromboxaneA2 (TA2) for secretion of granular contents. Thromboxane A2 is synthesized from arachidonic acid that is released from platelet phospholipids during the aggregation process under the influence of phospholipid (Baklaja *et al*., 2008).

1.2.1.3.4 The Role of Platelets in Hemostasis:

Two potent platelet aggregating agents are thrombin, which binds to Glycoprotein 5 (GPV) as well as to GPIb (vWF receptor), and collagen, which binds to GPIa/IIa. Thrombin and collagen can induce aggregation of platelets and secretion of platelet granular contents even if prostaglandin synthesis is blocked. A bond forms between fibrinogen and adjacent platelets through the interaction with platelet receptor complex GPIIb/IIIa. Fibrinogen binding only occurs after the platelet-activation-induced conformational change of the complex. GPIIb/IIIa is a transmembrane complex associated with actin on the inner surface of the platelet. Actin is a major component of platelet cytoskeleton. An actin-GPIIb/IIIa association is essential for clot retraction. Patients who show either the rare condition of a fibrinogenemia or lack GPIIb/IIIacomplex (Glanzmannthrombastenia) have poor clot retraction and hemorrhagic syndrome (Baklaja *et al*., 2008).

1.2.1.4 Coagulation cascade:

1.2.1.4.1 Classic Concept of the Coagulation Cascade:

The coagulation cascade is the sequential in vitro activation of coagulation factors following interaction with a foreign surface. Factors XII, XI, IX, X and II are intrinsic pathways factors which are converted to serine proteases and act on subsequent factors in the cascade; factors VIII and V are cofactors; the extrinsic pathway is activated by the interaction of factor VII and tissue factor. The activated partial thromboplastin time tests the intrinsic pathway; coagulation is initiated by contact with particulate matter such as kaolin and a 'partial thromboplastin' (such as cephalin) acts as a substitute for platelet phospholipid (Phl). The prothrombin time tests the extrinsic pathway,

coagulation being initiated by addition of a 'complete thromboplastin', which acts as a substitute for tissue factor. The thrombin time, in which thrombin is added to plasma, tests the final step of the common pathway, the conversion of fibrinogen to fibrin (Bain and Gupta, 2003).

1.2.1.4.2 Current Concept of the Coagulation Cascade:

In the current concept of the coagulation, the key initiating step is the exposure of TF to the circulation and reaction of TF with factor VIIa. The TF-factorVIIa complex can enzymatically activate factor X to Xa, factor IX to IXa, and factor XI to XIa. The initial activation of factor X to Xa may be important in getting the coagulation cascade started; however, a specific inhibitor produced by endothelium called tissue factor pathway inhibitor(TFPI) rapidly inactivates the TF-VIIa-Xa complex. Therefore, the major action of the TF-VIIa complex in vivo is the activation of factor IX to IXa, which then activates factor X to Xa. Activation of factor XI to XIa by the TF-VII a complex appears to play a relatively minor role in the coagulation cascade (Kern, 2002).

Activation of factor X to Xa and prothrombin (II) to thrombin (IIa) are key steps in the coagulation cascade since both Xa and thrombin have positive feedback activity on earlier steps of the cascade. Factor Xa activates VII to VIIa, increasing the amount of VIIa available to complex with TF (Kern, 2002).

Thrombin converts factor V to Va and factor VIII to VIIIa. It also activates factor XI to XIa and XIII to XIIIa. Thrombin is also a potent platelet agonist.

Factor X is activated by a complex of factor IXa, VIIIa, phospholipid, and calcium. Prothrombin is activated by a complex of factor Xa, Va, phospholipid, and calcium (Kern 2002).

1.2.1.4.3 Phases of Coagulation:

According to the cellular model, coagulation takes place in three stages: initiation, amplification, and propagation (Baklaja *et al*., 2008).

1.2.1.4.3.1 Initiation phase:

occurs through the binding of TF with F VIIa and activation of FX and F IX. Activated F X activates F V on the cell surface carryingTF, and this complex transforms a small quantity of prothrombin into thrombin (Baklaja *et al.*, 2008).

1.2.1.4.3.2 Amplification phase:

In the amplification stage, the created thrombin induces platelet activation, F V, F VIII, F XI and F XIII (Baklaja *et al.*, 2008).

1.2.1.4.3.3 Propagation phase:

During the propagation stage, FIXa and FVIIIa, located on the platelet surface, create a complex that strongly activatesF X. Activated F X and F Va, located on the platelet surface, create prothrombinase complex, which transforms a significant quantity of prothrombin into thrombin. Thrombin created in this way transforms fibrinogen into soluble fibrin, which under the influence of F XIIIa becomes insoluble coagulum. FXIa activates FIX and contributes to the additional formation of the thrombin surplus required for TAFI (thrombin activated fibrinolysis inhibitor) activation that ceases early fibrinolysis. This does not mean that the lack of FXI does not jeopardize coagulation, but, instead, compromises the inhibition of the early fibrinolysis, which contributes to a moderate bleeding risk (Baklaja *et al.*, 2008).

All coagulation factors are synthesized in the liver. Certain coagulation factors require the presence of vitamin K for their synthesis (Baklaja *et al.*, 2008).

1.2.1.4.4 Vitamine K dependant factors:

Vitamin K-dependent procoagulant factors II, VII, IX and X are synthesized in the liver, circulate as zymogens, are activated on the phospholipid surface and are limited by proteolysis. These factors belong to the serine proteases. Serine is found on the active site of the molecule's carboxyterminal. On the molecule's amino-terminal, each factor has 9 to 12 γ-carboxyglutamine residues, which are known as G1a domains, and are significant for binding calcium. Vitamin K is necessary for the carboxylation of these proteins. Inhibition of carboxylation

leads to formation of factors that are incapable of binding to phl and, thus, cannot manifest their Procoagulant activity (Baklaja *et al*., 2008).

Transition of prothrombin into thrombin is caused by the cleavage of two peptide bonds. This leads to the formation of prothrombin and thrombin enzyme fragments 1.2 that are markers for hemostatic activity (Baklaja *et al*., 2008).

1.2.1.5 Inhibition of coagulation:

Several factors support haemostasis by inhibition, limiting blood coagulation to the injured vessels and preventing thromboembolic complications. The most important inhibitors are antithrombin and protein C, including its cofactor protein S. Antithrombin has several important properties, including inhibition of coagulation and anti-inflammatory activity. Together with heparin sulphate and other glucosaminoglycans in vivo, and with heparin during treatment, antithrombin blocks thrombin and activated factors that circulate freely in blood vessels. This prevents coagulation in non-injured blood vessels and limits thrombin activation to the location of the injury. Protein C activates when free thrombin binds to thrombomodulin, an endothelial cell receptor. Activated protein C (APC) and its cofactor protein S inactivate FVa and FVIIIa, which subsequently inhibit the production of thrombin. In addition to anticoagulation, APC also has other properties, including anti-inflammatory and barrier-protective effects (Karlsson, 2014).

1.2.1.6 Fibrinolysis:

The final step of fibrinolysis is to dissolve the fibrin clot. Tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA)activate plasminogen to form plasmin. Plasminogen circulates freely in plasma, binding to fibrin on the clot. Activation results in local fibrinolytic activity adjacent to the clot. When fibrin dissolves, a number of different fragments form (e.g. D-dimers). Fibrinolysis inhibitors, e.g. plasminogen activator inhibitor 1 (PAI-1), plasminogen activator inhibitor 2 (PAI-2) and antiplasmin, provide protection from uncontrolled fibrinolysis. During pregnancy, trophoblasts in the placenta

produce PAI-2. PAI-1 and PAI-2 inhibit conversion of plasminogen to plasmin and antiplasmin inhibits plasmin. Tranexamic acid, a prohaemostatic drug, inhibits fibrinolysis by preventing the activation of plasminogen to plasmin (Karlsson, 2014).

1.2.1.7. Laboratory diagnosis of hemorrhagic disorders:

Diagnosis of hemorrhagic disorders can be based on:

-history of the disease.

-clinical check-up.

-laboratory test.

- Screening tests for vascular and platelets disorders.
- Screening tests for coagulation disorders.
- Specific tests (Baklaja *et al*., 2008).

Laboratory analysis:

In order to diagnose vascular and platelets disorders, bleeding time and platelet count should be determined.

Determination of bleeding time is the most easily and most frequently performed test. Normal values range from 1-3 minutes (Duke). It is prolonged in blood vessel wall structure disorder, decreased plts count, disorders of adhesion and aggregation, disturbance of plts release reaction.

Normal plts count ranges from 150-450x10^9/L.

Bleeding time can prolonged due to:

-abnormal vessel constriction.

-low platelet count.

-abnormal platelet adhesion.

-abnormal platelet release of ADP or TA2.

-Abnormal platelet aggregation.

-vWD.

TT,PT and PTT are determined in order to diagnose coagulopathies. These tests include disorders of intrinsic, extrinsic and common activation pathways, both

individual and associated defects of several coagulation factors (Baklaja *et al*., 2008).

Thrombin Time (TT):

Measures the time of fibrinogen transformation into fibrin under the influence of thrombin. Thus, all other factors are avoided. TT is prolonged in hypo- and afibrinogenemias, in the presence of inhibitors, when fibrinogen changes into fibrion, such as heparin, and in the presence of FDP and soluble fibrin monomers. Normal time ranges from 16-20 seconds (Baklaja *et al*., 2008).

Prothrombin Time (PT):

Determines extrinsic and common coagulation pathways and thus, is prolonged in deficient FII, FV, FVII,FX and fibrinogen. This test is necessary in oral anticoagulant therapy control, as well as in the investigation of liver function. (Baklaja *et al*., 2008).

 Normal values range from 11-16 seconds (Lewis *et al*., 2006).

Activated Partial prothrombin Time(APTT):

Determines the internal mechanism of blood coagulation and is prolonged in deficiencies of all plasma coagulation factors: FI, FII, FV, F VIII, FX, FXI and FXII, except in FVII and FXIII deficiency. It is prolonged in prekallikrein and HMWK deficiency.

APTT is prolonged in the presence of heparin and inhibitors. It is particularly prolonged in hemophilia and in the presence of heparin. This test has completely replaced the coagulation time test from the venous blood. A coagulation time test from capillary blood is not safe because of the presence of tissue thromboplastin; thus, it shows normal values in all coagulopathies, even in hemophilias (Baklaja *et al.,* 2008).

 Normal time ranges from 26-40 seconds (Lewis *et al*., 2006).

All tests are performed in plasma obtained by blood centrifugation, drawn in 3.8% sodium citrate in 1:9 ratio. The blood is drawn with plastic syringes into

plastic or silicon tubes. Tests are performed right after blood has been drawn (Baklaja *et al*., 2008).

1.2.2 Fibrinogen:

1.2.2.1 Fibrinogen structure:

The fibrinogen gene cluster is located on chromosome 4q31 in the order γ α β with β transcribed in the opposite direction to γ and α. The three chains of fibrinogen are disulphide cross linked and folded together in an intricate manner. The overall structure of fibrinogen is a symmetrical dimer α_2, β_2, γ_2. Viewed by electron microscopy the molecule is trinodular with the outer two globular domains(fragments D) containing the carboxy termini of three chains, connected to the central globular domain (fragment E) which contains the N-termini of all 6 chains lethered together by disulphide bonds. The lateral and central globular domains are connected by soiled coil regions, forming α-helical ropes. Polymerization of fibrinogen occurs when thrombin cleaves two short negatively charged fibrinopeptides A and B from the N-termini of the α and β chains respectively. This reveals new N-terminal sequences in the fragment E region (called knobs) which fit in to holes in the fragment D region. Polymerization when occurs spontaneously in a stggered half overlap arry, which can elongate indefinitely in either direction. The recently, published three dimensional structure of the fragment D region of fibrinogen as a dimer complexed with a synthetic knob peptide allows a molecular model of fibrin to be built. Completely confirming the structure proposed from electron microscopy and biochemical data (Hoffbrand *et al*., 2001). Increased in tissue inflammation or damage, acute infection, myocardial infarction, medications, oral contraceptives, pregnancy. Decreased in disseminated intravascular coagulation(DIC), primary or secondary fibrinolysis, liver disease, hereditary afibrinogenemia or hypofibrinogenemia and cachexia (Baklaja *et al*., 2008).

1.2.2.2 Fibrinogen sources:

Fibrinogen is a soluble plasma glycoprotein produced in the liver by the hepatocyte (Ibnouf, 2014).

It plays a critical role in achieving haemostasis during haemorrhage, but also acts as an acute phase protein (Karlsson, 2014).

The normal plasma concentration of fibrinogen is approximately 200 – 400 mg/dl. Normally, it is easy and fast to recreate fibrinogen from animals, which we get this protein from (White *et al*., 1973).

Increasing plasma concentration of fibrinogen are associated with an increased risk of stroke. The association is understandable because raised plasma fibrinogen could cause stroke by increasing plasma viscosity or by promoting thrombosis (Hankey, 2002).

Fibrinogen was firstly isolated from horse plasma by Hammarsten in 1876, although an inactive precursor to fibrin was proposed to exist as early as 1859 By Deni de Commery. Fibrinogen can undergo remarkable transformation from soluble monomers (fibrinogen) to an insoluble polymer gel(polymerized fibrin).

Fibrinogen is a 340 KD (kilo Dalton) glycoprotein that circulates in the plasma at a concentration of 2-4 g/L, with a half- life of 4 days (Ibrahim, 2010).

1.2.2.3 Fibrinogen measurement:

There are several assays for measuring fibrinogen levels in plasma. Most laboratories use and recommend the Clauss method , with a non-pregnant reference range of 2.0-4.5 g/L and coefficients of variation of 7% at 2 g/L and of 5% at 3 g/L. The Clauss method is a functional assay based upon the time of fibrin clot formation. The immunological assay measures fibrinogen antigen rather than functional fibrinogen. In patients with dysfibrinogenaemias, there is a discrepancy between functional and antigen levels . Studies with thromboelastography and thromboelastometry have shown associations between fibrinogen concentrations with their clot strength variables (Karlsson, 2014) . Fibrinogen has been studied since the mid-nineteenth century. During the

twentieth century, it has been used to treat bleeding, but interest in the substance declined due to the product sometimes was contaminated. Newer drugs have shown good efficacy. During pregnancy, the fibrinogen concentration increases progressively and remains high for approximately two weeks after delivery (Karlsson, 2014).

1.2.3 Pregnancy:

Pregnancy is the fertilization and development of one or more offspring, known as an embryo or fetus, in a woman' s uterus. It is the common name for gestation in humans. Childbirth usually occurs about 38 weeks after conception in women who have a menstrual cycle length of four weeks, this is approximately 40weeks from the start of the last normal menstrual period (LNMP). An embryo is the developing offspring during the first 8weeks following conception and subsequently the term fetus is used until birth (Ifeanyi *et al*., 2014). In many societies pregnancy is somewhat arbitrarily divided into three trimester periods, as a means to simplify reference to the different stages of prenatal development. The first trimester carries the highest risk of miscarriage. During the second trimester, the development of the fetus can be more easily monitored and diagnosed. The beginning of the third often approximates the point of viability or the ability of the fetus to survive, with or without medical help, outside of the uterus (Ifeanyi *et al*., 2014).

In the third trimester, the uterus expands making up a larger portion of the woman's abdomen. During the final stages of gestation before childbirth the fetus and uterus will drop to a lower position. For instance, the enlarged uterus may impede blood flow by compressing the lower pressured vena cava, with the left lateral positions appearing to providing better oxygenation to the infant (Ibnouf, 2014).

Pregnancy is associated with profound anatomical, physiological, biochemical and endocrine changes that affect multiple organs and systems. These changes are essential to help the woman to adapt to the pregnant state and to aid fetal

growth and survival. However, such anatomical and physiological changes may cause confusion during clinical examination of a pregnant woman. Similarly, changes in blood biochemistry during pregnancy may create difficulties in interpretation of results. Conversely, clinicians also need to recognize pathological deviations in these normal anatomical and physiological changes during pregnancy to institute appropriate action to improve maternal and fetal outcome (Chandraharan and Arulkumaran, 2012).

1.2.3.1 Physiology:

The most commonly used event to mark the initiation of pregnancy is the first day of the woman's last normal menstrual period, and the resulting fetal age is called the gestational age. This choice is a result of a lack of a convenient way to discern the point in time when the actual creation of the baby naturally happens. In case of in vitro fertilization, gestational age is calculated by days from oocyte retrival +14 days (Ibnouf, 2014).

1.2.3.2 Hormonal change:

Pregnant woman experience adjustment in their endocrine system. Levels of progesterone and estrogen rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle estrogen mainly produced by placenta and associated with fetal wellbeing. Woman also experience increased Human Chorionic Gonadotropin (HCG); which is produced by the placenta. Prolactin levels increase due to maternal pituitary gland enlargement by 50%. This mediate changes in the structure of the mammary gland from the ductal to lobulo-alveolar. Parathyroid hormone is increased which leads to increases of calcium uptake in the gut and reabsorption by the kidneys. Adrenal hormones such as cortisol and aldesterone also increased, human placental lactogen (HPL)is produced by placenta and stimulates lypolysis and fatty acids metabolism by the woman, conserving blood glucose for use by the fetus. It can also decrease maternal tissue sensitivity to insulin ; resulting in gestational diabetes (Koller *et al*., 1979)

1.2.3.3 Changes in the gastrointestinal system:

Nausea and vomiting are the most frequent complaints involving the gastrointestinal system and usually happen in early pregnancy while heartburn happen primarily in late pregnancy. The gums become hyperemic and edematous during pregnancy and tend to bleed. The muscular wall of the esophagus is relaxed and this may cause reflux, which in turn can lead to esophagitis and heartburn. The stomach and the intestines have decreased motility presumably due to the effect of progesterone on smooth muscle contractility. This causes an increase in the time that it takes for the stomach to empty. Reduced gastric secretion has also been documented and it could account for the improvement of peptic ulcers sometimes observed in pregnancy(Kofinas, 2015). Decreased motility of the large intestine may lead to constipation. The liver is affected significantly by pregnancy. Cholestatic jaundice is considered to be the result of estrogen effect on elimination of bilirubin by the liver. The effect of estrogens also, is to increase protein synthesis in the liver, which leads to increased production of fibrinogen and binding proteins. The liver enzymes are usually unaffected with the exception of alkaline phosphatase, which is increased at approximately twofold to four fold that is a result of a placental production. Pregnancy increases the size and decreases the motility of the gall bladder. The decreasing motility and increase in volume, combined with changes in the bile's composition, explain the correlation between the incidence of cholelithiasis and pregnancy (Kofinas, 2015).

1.2.3.4 Cardiovasular changes:

In brief, the cardiovascular changes involve a substantial change in the blood volume, cardiac output, heart rate, systemic arterial blood pressure, systemic vascular resistance, oxygen consumption and alterations in regional blood flow of various organ systems (Kofinas, 2015).

A slight decrease in the systolic arterial blood pressure and a significant decrease in the diastolic pressure have been observed to occur in normal pregnancy. This decrease becomes evident in the late first trimester and continues throughout most of the second trimester. The lowest values are noted in mid pregnancy and there after the blood pressure returns toward non-pregnant levels before term. The degree of change in the blood pressure parameters has been found to be affected by parity, smoking, preexisting hypertension, maternal age and ethnic background. In the typical normal pregnancy the mean arterial pressure(diastolic plus 1/3 of the difference between systolic and diastolic) is less than 85 mm of mercury. Studies have found that when the mean arterial blood pressure in the mid second trimester is higher than 90 mm of mercury, there is increased perinatal mortality and morbidity (Kofinas, 2015).

1.2.3.5 Hemostasis during pregnancy:

Hemostasis becomes significantly altered during pregnancy; estrogen causes most of the factors to increase. Nature thus reduces the risk of bleeding during childbirth, unfortunately also increasing the risk of thromboembolic complications. Some investigators have noted a decrease in platelet count, whereas others have noted no change. Seven percent of pregnant women develop gestational thrombocytopenia ranging from 70 to 150 x 109/L(Datta,2010).

In postpartum, there is a reactive increase in platelet count and normalization occurs within two months. Most coagulation factors increase, including fibrinogen, FVII, FVIII, FIX, FX and Factor XII (FXII). FII and FV remain unchanged, while FXI and FXIII decrease. The coagulation inhibition factors change; antithrombin declines slightly but remains within the non-pregnant reference range, protein C will be unchanged while protein S decreases by about 50%.Plasminogen increases, but PAI-1 and PAI-2 increase more, resulting in decreased fibrinolysis due to increased secretion by the placental cells.

Together, changes in haemostasis increase coagulation and decrease fibrinolysis, resulting in a hypercoagulable state, most likely entailing decreased risk of bleeding but increased risk of thromboembolic complications (Karlsson, 2014;Datta, 2010).

1.2.3.6 Correlation between fibrinogen and pregnancy:

There are significant increases in the production of several procoagulant factors and a reduction in plasma fibrinolytic activity. Marked increase in plasma fibrinogen concentration. The need for relative hypercoagulability is particularly apparent at the time of placental separation. At term, about 500-700 ml of blood flows through the placental bed per minute. Without effective and rapid hemostasis, a woman could die from exsanguinations within few minutes. Fibrin begins to be deposited over the placental site and ultimately between 5 and 10 percent of all the fibrinogen in the circulation is used up for this purpose. Factors that impede this haemostatic process, such as inadequate uterine contraction or incomplete placental separation, can therefore rapidly lead to depletion of fibrinogen reserve (Baker, 2006)

1.2.4 Hypertension:

1.2.4.1 Definition:

Hypertension(HTN) or high blood pressure, is a chronic medical condition in which the blood pressure in the arteries is elevated. Blood pressure is summarized by two measurements, systolic and diastolic, which depend on whether the heart muscle is contracting(systole) or relaxed between beats(diastole). This equals the maximum and minimum pressure respectively. Normal blood pressure at rest is within the range of 100-140 mmHg systolic(top reading) and 60-90 mmHg diastolic (bottom reading). High blood pressure is said to be present if it is often at or above 140/90mmHg (Carretero and Oparil 2000)

Hypertensive disorders during pregnancy occur in women with pre-existing primary or secondary chronic hypertension, and in women who develop new-onset hypertension in the second half of pregnancy (NICE 2010).

1.2.4.2 Classification of Hypertensive Disorders during Pregnancy:

Four categories:

1. Preeclampsia-eclampsia (BP elevation after 20 weeks of gestation with proteinuria or any of the severe features of preeclampsia)

2. Chronic hypertension (of any cause that predates pregnancy)

3. Chronic hypertension with superimposed preeclampsia (chronic hypertension in association with preeclampsia)

4. Gestational hypertension (BP elevation after 20 weeks of gestation in the absence of proteinuria or any of the severe features of preeclampsia) (Robert *et al*.,2013).

Pregnancy is a physiological process in women but it may be associated with certain risks to the health and life of both the mother and child. Hypertensive disorders complicating pregnancy are common and form one of the deadly triad along with hemorrhage and infection that results in maternal and perinatal morbidity and mortality. Pregnancy can induce hypertension in normotensive women or aggravate already existing hypertension or appears for the first time during pregnancy. The identification of this clinical entity and effective management play a significant role in the outcome of pregnancy, both for the mother and the baby. In the developing countries with uncared pregnancy, this entity on many occasions remains undetected till major complications supervene (Naaz *et al.,* 2015).

Preeclampsia is a complication of pregnancy constituting a major cause of maternal and fatal morbidity and mortality. Pregnancy is a hypercoagulable state with changes in procoagulant, anticoagulant, and fibrinolytic systems. In preeclampsia, there is a shift in the haemostatic balance towards a pro-thrombotic state, together with changes in endothelial function. It is a state of

enhanced coagulation as evidenced by an increased amount of clotting factors in maternal circulation (Alwan *et al*., 2013).

Chronic hypertension in pregnancy is defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic pressure before pregnancy or, for women who first present for care during pregnancy, before 20 weeks of gestation. The prevalence of chronic hypertension in pregnancy in the United States is estimated to be as high as 3%1 and has been increasing over time. This increase in prevalence is primarily attributable to the increased prevalence of obesity, a major risk factor for hypertension, as well as the delay in childbearing to ages when chronic hypertension is more common(Seely and Echer, 2011). Therefore, an increasing number of women enter pregnancy with hypertension and need both counseling regarding the risks of chronic hypertension in pregnancy and adjustment of antihypertensive treatment before and during pregnancy. Most women with chronic hypertension have good pregnancy outcomes, but these women are at increased risk for pregnancy complications, as compared with the general population. The risk of an adverse outcome increases with the severity of hypertension and end-organ damage(Seely and Echer, 2011). Furthermore, some antihypertensive agents carry risks in pregnancy and should be discontinued before conception. Since nearly 50% of pregnancies in the United States are unplanned, it is important to counsel women of reproductive age who have hypertension regarding such risks as part of routine care. Women with chronic hypertension have an increased frequency of preeclampsia, as well as placental abruption, fetal growth restriction, preterm birth, and cesarean section. The risk of superimposed preeclampsia increases with an increasing duration of hypertension. Preeclampsia is a leading cause of preterm birth and cesarean delivery in this population. Women with chronic hypertension with superimposed preeclampsia are at increased risk for giving birth to an infant who is small for gestational age and for placental abruption, as

compared with women with chronic hypertension without super imposed preeclampsia (Seely and Echer, 2011).

Most women with chronic hypertension have a decrease in blood pressure during pregnancy, similar to that observed in normotensive women; blood pressure falls toward the end of the first trimester and rises toward pregnancy values during the third trimester. As a result, antihypertensive medications can often be tapered during pregnancy. However, in addition to the subset of women with chronic hypertension in whom preeclampsia develops, another women have worsening of hypertension during pregnancy without the development of preeclampsia (Seely and Echer, 2011).

1.2.4.3 Management of hypertension:

One way to reduce the impact of arterial hypertension on maternal mortality is to establish the correct diagnosis of hypertensive disorders of pregnancy, and to proceed with an early intervention when it is diagnosed. The clinical signs are considered to be a late manifestation of a disease that has been present since the first trimester of gestation due to "Diagnostic delay". Many tests have attempted to establish the diagnosis of preeclampsia as early as possible, often even before the patient present arterial hypertension(Naaz *et al.,* 2015). Tests reported for the early diagnosis of hypertensive disorders are Doppler ultrasound assessment of maternal and fetal circulation, uric acid concentration, the supine pressure test, the angiotensin test, microalbuminuria, plasma fibronectin concentration, plasma antithrombin activity, calciuria, prothrombin time, platelet count, fibrinogen levels, APTT and other tests, all of which are of debatable efficacy and practicality (Naaz *et al*., 2015).

1.2.4.4 Fibrinogen and preeclampsia:

Preeclampsia (PE) being by itself a highly thrombotic and procoagulant state with platelet activation and consumption, promoting of thrombin and fibrin formation with destruction. The state of enhanced coagulation in preeclampsia was evidenced by elevated level of von Willebrand's factor, increased the

concentration of total fibrinogen and the percentage of high molecular weight fibrinogen in preeclampsia as the activated maternal vascular endothelium also triggers a generalized intravascular inflammatory reaction. Plasma levels of fibrinogen vary according to both individual and inflammatory parameters. During normal pregnancy the plasma levels of fibrinogen increase, and in women with preeclampsia the fibrinogen levels are even higher. In addition to its importance for clot formation, fibrinogen also interacts with angiogenic factors such as fibroblast growth factor-2 and vascular endothelial growth factor (VEGF). It also binds to histidine-rich glycoprotein (HRG) (Alwan *et al.*, 2013).

1.2.5 Diabetes mellitus (D.M):

1.2.5.1 Definition:

Diabetes is a chronic disease that occurs either when the pancreas doesn't produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood glucose. Hyperglycemia, or raised blood glucose, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels (WHO, 2013)

Diabetes mellitus has long been considered a disease of minor significance to world health, but is now developing into one of the main public health challenges for the 21st century. The past two decades have seen an explosive increase in the number of people diagnosed with diabetes mellitus worldwide(Venkataramana, 2013).

This diabetes mellitus epidemic relates particularly to type II diabetes, which is taking place both in developed and developing countries (Venkataramana, 2013).

1.2.5.2 Types of diabetes mellitus:

Diabetes mellitus can be divided into:

1.2.5.2.1 Type 1diabetes:

Formerly known as Insulin Dependent Diabetes Mellitus (IDDM), characterized by hyperglycemia due to an absolute deficiency of the insulin hormone produced by the pancreas. Patients require lifelong insulin injection for survival and are at increased risk of developing microvascular and macrovascular complications (WHO, 1999).

1.2.5.2.2 Type 2 diabetes (T2D):

Type II diabetes is characterized by insulin resistance and/or abnormal) insulin secretion. Individuals with type II diabetes are not dependent on exogenous insulin, but may require it for control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. It has been hypothesized that atherosclerotic cardiovascular disease and type 2 diabetes arise from a "common soil" and chronic inflammation may be such a candidate. Inflammatory markers, such as high white cell count, high fibrinogen, or low albumin. In type 2 diabetes, fibrinogen production is increased both in the post absorptive state and in response to hyperinsulinemia. In type 2 diabetic patients, post absorptive albumin synthesis and its response to insulin were normal, where as fibrinogen synthesis was increased, irrespective of metaboliccontrol. Fibrinogen , serum Total protein, of longterm type-2 diabetics are significantly elevated. In patients with non insulin dependent diabetes mellitus, high plasma levels of C-reactive protein and fibrinogen are present (Venkataramana, 2013).

1.2.5.2.3 Gestational diabetes:

Characterized by hyperglycemia of varying severity diagnosed during pregnancy (without previously known diabetes) and are usually (but not always) resolving within 6 weeks of delivery. Risk to pregnancy itself include congenital malformations, birth weight and an elevated risk of perinatal mortality. Increased risk to woman of developing diabetes (T2D) later in life. Themechanism is not completely understood but hormones of pregnancy appear to interfere with insulin action (WHO, 1999).

1.2.5.3 Complications of diabetes:

diabetes complications are divided into microvascular (due to damage to small blood vessels) and macrovascular (due to damage to large blood vessels).

Microvacular complications include damage to the eyes (retinopathy) leading to blindness, to kidney (nephropathy) leading to renal failure and to nerves (neuropathy) leading to impotence and diabetic foot disorders.

Macrovascular complications include cardiovascular diseases such as heart attacks, strokes and insufficiency in blood flow to legs (WHO, 1999).

Diabetic and hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics. This study is aimed to evaluate some biochemical markers in hypertensive and diabetic disorders of pregnancy which includes: PT, APTT and Fibrinogen level.

1.2.6 Previous studies:

Increased in fibrinogen are evident in early pregnancy where as fibrinolysis, perhaps in response to the Procoagulant environment of pregnancy, is increased during late pregnancy. Before development of clinically overt hypertension, this consistent with altered endothelial function with preeclampsia that may contribute to, or reflect, the vasoculpathy accompanying this disorder(Hale *et al.,* 2012).

Fibrinogen level was found to be higher in preeclampsia and normal pregnant than non –pregnant , also significant prolongation of PT was seen in preeclampsia when compared to non- pregnant (Anuradha, 2015).

Changes in coagulation profile that occur in normal pregnancy includes the biochemical adaptation especially the hematological changes that occur in response to pregnancy are profound. The level of several blood coagulation factors are increased during pregnancy. Plasma fibrinogen concentration increases about 65% late in pregnancy(Naaz *et al.,* 2015).

Coagulation among Sudanese pregnant women in police hospital – Khartoum sudan , showed significant decrease in PT , APTT and also there was

significant increase in plasma fibrinogen level in pregnant women when compared with non pregnant (Elsharif, 2013).

1.3. Rationale:

Pregnancy is a hypercoagulable state in itself. The determination of the level of plasma fibrinogen in pregnant women is very important in the diagnosis of disease and follow up of pregnancy. Since arterial disease is the major underlying factor leading to the most clinically relevant cardiovascular events and these events are usually due to formation of thrombus at the site of an atherosclerotic plaque, this research was done to detect the possibility of thrombosis in hypertensive and diabetic pregnant women in Sudan.

1.4. Objectives:

1.4.1. General objectives:

To measure thrombophilia profile in hypertensive, diabetic and normal pregnant women.

1.4.2. Specific objectives:

1- To measure prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen level in hypertensive, diabetic and normal pregnant females.

2- To compare PT,APTT and fibrinogen level between hypertensive pregnant, diabetic pregnant and normal pregnant with non pregnant females.

3- To determine the effect of both diseases in PT, APTT and fibrinogen level.

CHAPTER TWO

MATERIALS AND METHODS

Materials and Methods

2.1 Materials:

2.1.1 Study design:

It was analytical case-control study conducted in Khartoum State in Alsuodi, Alban Gadeed and East Nil Model hospital during the period from September 2015 to December 2015. To measure the PT, APTT and the plasma fibrinogen level in hypertensive pregnant, diabetic pregnant and normal pregnant women.

2.1.2 Study population:

First ninety pregnant women (case group) were enrolled in this study. Thirty of them with hypertension , thirty were diabetic pregnant and thirty were normal pregnant. Second thirty apparently healthy non- pregnant women with age matches (control group).

2.1.3 Inclusion criteria:

Pregnant women at third trimester with hypertension , diabetes pregnant and normal pregnant women.

Healthy non-pregnant were the control group.

2.1.4 Exclusion criteria:

Presence of any history related to high or low fibrinogen and patient under anticoagulation therapy were also excluded.

2.1.5 Sample collection:

Pregnant women were diagnosed as hypertensive, diabetic and non diseased were selected and data collection using self administrated questionnaire which specifically designed to obtain information that help in study.

2.1.6 Ethical consideration:

The study was conducted after permission from the institutional ethical committee. Written and verbal consent of cases and controls were taken.

2.1.7 Blood collection and preparation:

2.7 ml of citrated anti coagulated venous blood samples were collected (9 part blood to 1 part anti coagulant). Blood was thoroughly mixed with the anti coagulant. Samples were centrifuged at 3000rpm for 15 minutes to obtain platelet-poor plasma (PPP). Plasma was separated from cells into eppendorf tube and tested.

2.2 Methodology:

2.2.1 Principle of coagulometer (clot instrument-DiaMed):

The coagulometer clot analyzer has an optical measurement system which detects sudden variation in optical density when a clot is formed. The chronometer and the stirring system are activated by a sudden change of the optical density. This permits the initiation of the time measurement when the sample is added to the reagent and stop the measurement time at the moment the clot is formed. The system has a programmable security time during which variations in the optical density, when the reagent and the plasma are still in the homogenization phase, cannot activate the detection cell.

The continues mixing is guarantees a perfect homogenization and make the measurement possible of low concentration of fibrinogen by grouping the fibrin filament in the center of the optical pass.

2.2.2 procedure of coagulometer:

Cuvette was placed corresponding to the determination that were done on the thermostat. A magnetic stirrer was installed in every Cuvette and waited for the instrument to reach 37ºc after that into the Cuvette , the sample or reagent volume required were introduced.

PT= (200µl of reagent).

APTT=(100µl of reagent + 100µl of plasma).

Fibrinogen =(200µl of diluted plasma).

When thermo station time was finished the Cuvette was placed on reading well, the chronometer was remained inactive for some seconds and when it was

showed 000,0 at this moment the reagent or plasma was added with disposable tip pipette. The liquid was left to get down with one below and all the reaction was started at the same time.

One hundred micro liter of starter was added:

PT= plasma

APTT= cacl$_2$

Fibrinogen= thrombin reagent.

When the reagent and plasma were in contact and optical density variation were produced that automatically activate the digital chronometer and the magnetic mixer. When the clot was start to formed and optical density was produced and stopped the chronometer and mixer the clotting time appears on the display.

2.2.3 Measurement of fibrinogen:

2.2.3.1 Principle:

The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

2.2.3.2 Reagents:

Thrombin reagent: which was a lyophilized preparation from bovine source ~ 50 NIH unit per vial.

Fibrinogen calibrator: which was a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on a mg /dl basis (refer FIBROQUANT graph paper supplied with each kit for the value of each clot).

Owren's buffer: ready to use (pH 7.35).

2.2.3.3 Procedure:

The reagents and samples were warmed at room temperature before testing.

A: Procedure For Fibrinogen Calibration Curve Preparation:

1. The FIBROQUANT thrombin reagent vial was reconstituted exactly with 1.0 ml of distilled water, waited for 5 minutes, gently swirled till the solution

attained homogeneity. Further the vial was kept for 10 minutes to attain equilibrium. Once reconstituted it is ready to use for the fibrinogen test.

2. The FIBROQUNT fibrinogen calibrator vial was reconstituted exactly with 1.0 ml of distilled water, waited for 5 minutes, and the vial was swirled gently till the solution attained homogeneity. The vial was further kept for 10 minutes to attain equilibrium. This is the fibrinogen calibrator stock solution.

3. The fibrinogen calibrator stock solution was diluted with Owren's buffer as follows:

Test tube No	I	II	III
Owren's buffer	NIL	800µl	900µl
Fibrinogen calibrator	200µl	200µl	100µl
Dilution calibrator	NIL	1:5	1:10

1. Two hundred (200µl) of each fibrinogen calibrator was pipetted into clean test tube and pre warmed for 3 minutes at 37ºc .

2. 100µl of reconstituted thrombin reagent was added (pre warmed at 37ºc for one minute) and Fibrinogen level was measured by clauss method. A calibration curve was prepared by preparing serial dilutions of calibration plasma with Owre's veronal buffer (1 in 5, 1 in 10, 1 in 20, and 1 in 40). 0.2 ml 0f each dilution was warmed to 37ºc for 3 minutes, then 0.1 ml of bovine thrombin solution was added, and the clotting time was measured by using coagulometer. Each test was performed in duplicate, the average was calculated and a calibration curve was conducted (the clotting time in seconds against the fibrinogen concentration in mg/dl) on log/log graph paper. The 1 in 10 dilution was considered to be 100% fibrinogen concentration. 1 in 10 dilution was made from each patient's plasma, thrombin time was measured as mentioned above, also in duplicate, and the fibrinogen level was determined in mg/dl from the calibration curve.

Normal range:

The normal range from 180 to 360 mg/dl (Lewis *et al*., 2006).

Quality control:

Test done in duplicate .

Correct mixture of blood .

Plasma and reagent warmed sufficiently.

Control of run done by using calibrator with known concentration.

Good storage of reagent.

2.2.4 Prothrombin time:

2.2.4.1 Principle:

This tests the extrinsic pathway. It involves the addition of brain Thromboplastin and calcium chloride to platelet- poor plasma (PPP) and observe the formation of clot and record the time in seconds.

2.2.4.2 Reagents:

Calcium-Thromboplastin (rabbit brain) liquid with $CaCl_2$ (0.025 mol/l).

2.2.4.3 Sample:

Platelet poor plasma was prepared by centrifugation of citrated plasma at 3000 rpm for 15 minutes and the plasma separated from the cells in eppendorf tube and tested.

2.2.4.4 Procedure:

One hundred micro liter of plasma was warmed at 37ºc for 5 minutes. At the same time the thrombin reagent was warmed. Then two hundred micro liter of thrombin reagent was added to the warmed plasma in the reaction area, then the analyzer read the clotting time of PT and displayed the result in second.

2.2.5 Activated partial Thromboplastin time:

2.2.5.1 Principle:

Measurement the intrinsic pathway. The essential reagent is phospholipid substitute for platelet factor III, and contact activation is achieved by adding an

activator such as kaolin. These reagent added to the PPP and observe the formation of the clot and record the time in seconds.

2.2.5.2 procedure:

One hundred micro liter of test plasma was warmed at 37ºC for 3minutes. At the same time the APTT reagent and cacl$_2$ were also simultaneously incubated. Then 100µl of APTT reagent was added to the warmed plasma and mixed and again incubated at 37ºC for 3 minutes. After that 100µl of pre warmed cacl$_2$ was added. Then the analyzer read the clotting time and displayed the results in seconds.

2.2.6 statistical analysis:

Data were processed and analyzed using statistical package for social sciences (SPSS) version 16. T-Test, ANOVA and correlations were used to calculate P. value. Differences were considered statistically significant when *P. value* $\leq$ 0.05.

CHAPTER THREE

RESULTS

Chapter Three

3.Results

This study included 120 samples divided as followed; 30 samples from hypertensive pregnant, 30 samples from diabetic pregnant, 30 samples from pregnant have no diseases and 30 samples from healthy non pregnant women calculated men age of each, Table (3-1).

The results showed that the hypertensive pregnant has mean of prothrombin time (PT) of 13.82±2.10 sec, where as the control group showed mean PT of 15.48±2.65 sec with *p. value* =0.010 (by the independent sample test), the mean of APTT was 34.82±3.48 sec, where as the control APTT was 40.38±4.79 sec with *p. value*=0.000 and the mean of fibrinogen level was 520.23±68.05 mg/dl where as the mean in control group was 268.37±59.67 mg/dl with *P.value*= 0.000 ,Table (3-2).

Also the result showed that the diabetic pregnant has mean PT of 12.61±1.37sec, when compared with control group the *p. value* was = 0.000, the mean of APTT was 35.860±4.83 sec, with *p. value*=0.000 and the mean of fibrinogen level was 548.83±99.71 mg/dl with *p. value* = 0.000 ,Table (3-3).

Normal pregnant showed mean PT of 15.01±1.94sec, when compared with control group the *p. value* = 0.434, the mean of APTT was 40.88±2.83 sec, with *p. value*=0.491, and the mean of fibrinogen level was 398.23±38.39 mg/dl with *p. value*= 0.000 ,table (3-4).

When comparing the PT of the hypertensive pregnant with diabetic pregnant , normal pregnant and with control group found that the *P. value* was (=0.026, = 0.029 and =0.002) respectively, also the *P.value* of diabetic pregnant with normal pregnant was= 0.000, with control group was =0.000 and the *P.value* between normal pregnant and control group was= 0.378. Table(3-5).

When comparing the PTT in hypertensive pregnant with diabetic pregnant , normal pregnant and with control group found that the *P. value* was (=0.236, =

0.000 and =0.000) respectively, also the *P.value* of diabetic pregnant with normal pregnant was= 0.000, with control group was = 0.000 and the *P.value* between normal pregnant and control group was= 0.657. Table(3-6).

When comparing the fibrinogen level in hypertensive pregnant with diabetic pregnant , normal pregnant and with control group found that the *P. value* was (=0.116, = 0.000 and 0.000) respectively, the *P.value* of diabetic pregnant with normal pregnant was= 0.000, with control group was = 0.000 and the *P.value* between normal pregnant and control group was= 0.000. Table(3-7).

A weak but significant positive correlation was observed between the duration of diabetes and PT (r= + 0.361, *p. value* = 0.050)figure (3-1).

There was no significant correlation between the duration of diabetes and PTT (*P.value* =0.114) and also no significant correlation with fibrinogen level (*P. value*=0.50) and also, no significant correlation between the duration of hypertension and PT (*P. value*=0.165), PTT(*P.value*=0.486) and fibrinogen level (*P.value*=0.408).

Table(3-1): Base line characteristics of the study population:

Study groups	Mean ± SD		
	Age/years	Duration of disease/years	No of pregnancy
Hypertensive pregnant (N= 30)	28.97±6.14	3.10±2.16	4.07±2.97
Diabetic pregnant(N=30)	31.03±5.55	4.37±4.30	4.13±1.92
Normal pregnant (N=30)	26.97±6.80	-	3.60±2.47
Control (N=30)	28.03±7.44	-	-

Table(3-2):Comparison of PT between study population:

Study population	PT(mean ± SD)/sec	*p.value*
HTN pregnant(N=30)	13.823±2.107	0.000
Diabetic pregnant(N=30)	12.613±1.371	0.000
Normal pregnant(N=30)	12.613±1.371	0.434
control(N=30)	15.483±2.656	-

∗The mean difference is significant at ≤0.05 level.

∗HTN=hypertensive

Table (3-3):Comparison of APTT between study population:

Study population	APTT(mean ± SD)/sec	*p.value*
HTN pregnant(N=30)	34.283±3.48	0.000
Diabetic pregnant(N=30)	35.600±4.83	0.000
Normal pregnant(N=30)	40.887±3.82	0.491
control(N=30)	40.395±4.79	-

*The mean difference is significant at ≤ 0.05 level.

Table (3-4):Comparison of plasma Fibrinogen level between study population:

Study population	Fibrinogen(mean ± SD)/mg/dl	*p.value*
HTN pregnant(N=30)	520.23±68.05	0.000
Diabetic pregnant(N=30)	548.83±99.71	0.000
Normal pregnant(N=30)	398.23±38.39	0.000
control(N=30)	268.37±59.67	-

*The mean difference is significant at ≤ 0.05 level.

Table (3-5):Comparison of PT between study population groups:

Dependent variable	Study population(I)	Study population(II)	Mean of (I) ±SD	Mean of (II) ±SD	P.value
PT (sec)	HTN pregnant	diabetic pre	13.823±2.10	12.613±1.37	0.026
		Normal pre		15.010±1.94	0.029
		control		15.483±2.65	0.002
	diabetic pregnant	HTN pre	12.613±1.37	13.823±2.10	0.026
		normal pre		15.010±1.94	0.000
		control		15.483±2.65	0.000
	Normal pregnant	HTN pre	15.010±1.94	13.823±2.10	0.029
		Diabetic pre		12.613±1.37	0.000
		control		15.483±2.65	0.378
	control	HTN pre	15.483±2.65	13.823±2.10	0.002
		Diabetic pre		12.613±1.37	0.000
		Normal pre		15.010±1.94	0.378

*Key: HTN pre: hypertension pregnant women, diabetic pre: diabetic pregnant women, Normal pre: Normal pregnant women.

*The mean difference is significant at ≤0.05 level.

Table (3-6):Comparison of APTT between study population groups:

Dependent variable	Study population(I)	Study population(II)	Mean of (I) ±SD	Mean of (II) ±SD	P.value
APTT(sec)	HTN pregnant	diabetic pre	34.283±3.48	35.600±4.83	0.236
		Normal pre		40.887±3.82	0.000
		control		40.395±4.79	0.000
	diabetic pregnant	HTN pre	35.600±4.83	34.283±3.48	0.236
		normal pre		40.887±3.82	0.000
		control		40.395±4.79	0.000
	Normal pregnant	HTN pre	40.887±3.82	34.283±3.48	0.000
		diabetic pre		35.600±4.83	0.000
		control		40.395±4.79	0.657
	control	HTN pre	40.395±4.79	34.283±3.48	0.000
		diabetic pre		35.600±4.83	0.000
		Normal pre		40.887±3.82	0.657

∗ Key: HTN pre: hypertension pregnant women, diabetic pre: diabetic pregnant women, Normal pre: Normal pregnant women.

*The mean difference is significant at ≤0.05 level.

Table (3-7): Comparison of plasma Fibrinogen between study population groups:

Dependent variable	Study population(I)	Study population(II)	Mean of (I) ±SD	Mean of (II) ±SD	P.value
Fibrinogen (mg/dl)	HTN pregnant	Diabetic pre	520.23±68.05	548.83±99.71	0.116
		Normal pre		398.23±38.39	0.000
		control		268.37±59.67	0.000
	diabetic pregnant	HTN pre	548.83±99.71	520.23±68.05	0.116
		normal pre		398.23±38.39	0.000
		control		268.37±59.67	0.000
	Normal pregnant	HTN pre	398.23±38.39	520.23±68.05	0.000
		diabetic pre		548.83±99.71	0.000
		control		268.37±59.67	0.000
	control	HTN pre	268.37±59.67	520.23±68.05	0.000
		diabetic pre		548.83±99.71	0.000
		Normal pre		398.23±38.39	0.000

∗ Key: HTN pre: hypertension pregnant women, diabetic pre: diabetic pregnant women, Normal pre: Normal pregnant women.

*The mean difference is significant at ≤0.05 level.
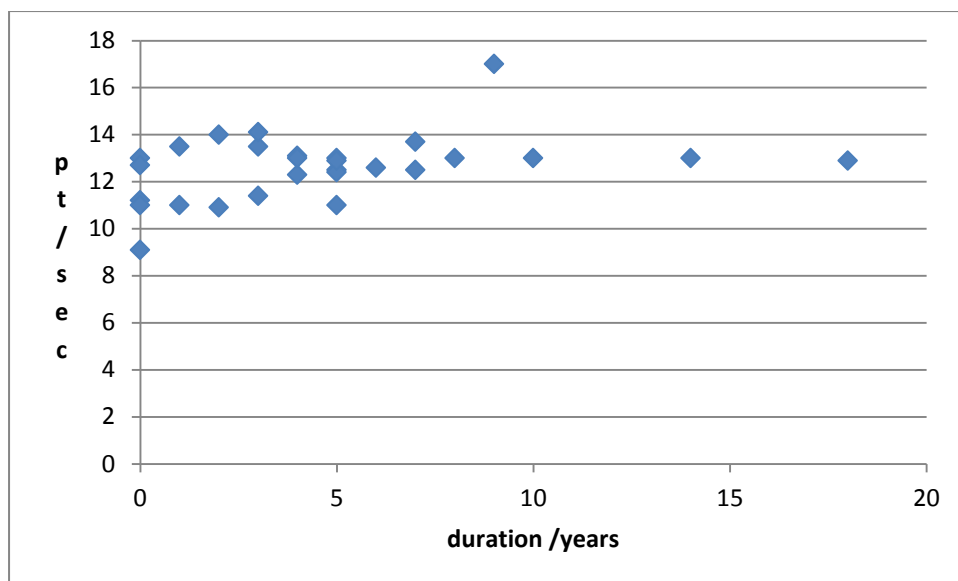
Figure(3-1):Correlation between duration of diabetes and PT.

Weak positive correlation (p. value =0.050, r =0.361).

CHAPTER FOUR

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4. Discussion, Conclusion and Recommendations

4.1 Discussion

This study was conducted in Khartoum State during the period from September to December 2015, to measure the PT, APTT and plasma fibrinogen level in hypertensive, diabetic and normal pregnant women.

The results of prothrombin time (PT) showed significantly lower value in pregnant with hypertension than the control (p. valve <0.05), although Eltahir (2012) found that there was insignificant increased values. Although PT was significantly lower in diabetic pregnant than the controls (p. valve <0.05). No significant difference between normal pregnant and control group (p. valve >0.05) the similar results were found by Mohamed (2010), while Abdeen (2012) and Ali (2015) found significant increased, where as Khairi (2011) and Elsharif (2013) found significant decreased. There was significantly lower values in diabetic pregnant than hypertensive pregnant (p. valve <0.05) and significantly lower values in hypertensive pregnant than normal pregnant (p. valve <0.05). this result disagreed with the result of Anuradha (2015) who was found significant prolongation in PT. Also found significant lower values when compared diabetic pregnant with normal pregnant (p. value<0.05) .The results from our study showed PT to be significantly lower in pregnant who had diabetes and hypertension than those who had no disease this may be due to the changes in coagulation and fibrinolytic system- which include factors (I,II, VII,VIII,IX and XII).

The result of activated partial Thromboplastin time (APTT) showed significantly lower value in pregnant with hypertension than the control (p. valve <0.05) while Eltahir (2012) disagreed with our result. Significantly a lower value in diabetics than controls (p. value<0.05). Although APTT showed no significant value in pregnant had no disease than the control group (p. value >0.05) While Mohamed (2010), Abdeen (2012) and Ali (2015) found that the

APTT was significantly increased. Khairi (2011) and Elsharif (2013) found significant decreased values. There was no significant difference between hypertensive pregnant and diabetic pregnant (p valve >0.05) , also found significant lower values in hypertensive pregnant compared to normal pregnant (p. valve >0.05) and significantly lower value in diabetic pregnant than normal pregnant(p. valve <0.05).The results from our study showed APTT to be significantly lower in pregnant who had diabetes and hypertension than those who had no diseases. However factor VIII,IX and XII activities increased gradually as pregnancy progress reached maximum values in the third trimester. The study showed fibrinogen to be significantly higher in pregnant with hypertension than the control (p. valve <0.05).The same study done in Sudanese patients by Eltahir (2012),who found that the mean of fibrinogen had been significantly increased in women with preeclampsia compared to normal pregnant. This firstly, because of preeclampsia is a systemic inflammation and fibrinogen is acute phase reactant, and increase in response to inflammation , the second factor, in healthy women fibrinogen level are increased by inflammation however , since compensatory coagulation and fibrinolysis become exaggerated in preeclampsia, consumption coagulopathy occurs and fibrinogen level are returned to normal values after the delivery. Naaz *et al.,* (2015), and   Alwan *et al.,* (2013) found similar result, also the plasma fibrinogen level was show significantly higher values in the diabetic pregnant and control group (p valve <0.05). This result disagreed with the result of Bronisz *et al.,*(2008) they found that statistically significant increase in fibrinogen level in diabetic pregnant in third trimester compared to the first trimester , the same result in normal pregnant, but there was no change when compare the diabetic with normal pregnant. The mean plasma fibrinogen level in normal pregnant was significantly higher than control (p. value <0.05)same study was done in Sudanese pregnant by Ibnouf (2014) who found similar results. The results agreed also with previous studies of Khairi (2011) , (Adler

G, *et al*.,(2000), Abdeen (2012), Elsharif (2013)and Ali (2015) found similar results . The higher fibrinogen concentration in third trimester which was mainly due to the inflammatory state of pregnancy and also fibrinogen being an acute phase protein.

Our study also showed fibrinogen to be significantly higher in hypertensive pregnant than normal pregnant (p. valve <0.05), Anuradha (2015) and Hale *et al*., (2012) found similar results. Also there was significant higher values in diabetic pregnant than normal pregnant (p.value <0.05), Bekdemir *et al*., (2015)showed similar results. No significant difference between pregnant who had diabetes and hypertension (P. valve >0.05). The results from our study showed fibrinogen to be significantly higher in pregnant with diabetes and hypertension than those who had no disease.

No significant correlation between duration of diabetes mellitus and plasma fibrinogen level (p. value > 0.05). There was weak positive correlation between duration of diabetes mellitus and PT (p. value < 0.05) and no significant correlation between duration of diabetes mellitus and APTT (p. value > 0.05).

There was no significant correlation between duration of hypertension and fibrinogen level (p. value > 0.05)and also, no significant correlation between duration of hypertension and PT (p value > 0.05).No significant correlation between duration of hypertension and APTT (p value > 0.05).

4.2 Conclusion:

Pregnant women with hypertension and diabetes were found to have shorter PT and APTT and increased level of plasma fibrinogen as compared to the control. Healthy pregnant women were found to have no difference in PT and PTT level and increased level of plasma fibrinogen as compared to the control. Hypertension pregnant women showed longer PT, shorter APTT and slight deceased fibrinogen level compared with diabetic one, while normal pregnant showed longer PT,APTT with decreased plasma fibrinogen level compared diabetic one.

Healthy pregnant women were found to have relatively longer PT and APTT and decreased level of plasma fibrinogen level compared to hypertensive one.

In conclusion, measurement of PT, APTT and fibrinogen level may be of some benefit in detecting thrombosis, which appears to complicate the hypertension and diabetes mellitus especially in pregnant women.

4.3 Recommendations:

1- PT, APTT and fibrinogen level should perform during pregnancy period as cardiovascular risk factor.

2- Thrombin generation , D-dimer , t.PA antigen , PAI-1 antigen and protein C should be performed in further studies.

3- Clinical and medical strategists should be adopted to follow up hemostatic changes in pregnancy.

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APPENDIXES

Collage of graduate studies- department of hematology

Questionnaire: coagulation profile in diabetic pregnant, hypertensive pregnant and normal pregnant women

Name:

Age:

Residence:

Tribe:

Telephone:

History of coagulopathy disease: Yes   NO

If you have diabetes: Yes   NO

Duration of disease   treatment use:

If you have hypertension: Yes   NO

Duration of disease: treatment use:

Control group: contraceptives use: Yes   NO

If yes what type:

Results:

1.PT ………… seconds   control……seconds

2.APTT……… seconds   control …..seconds

3. fibrinogen level ……mg/dl

Signature…………..   date ………………

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كليه الدراسات العليا

إبراء ذمه

نتعهد بان الهدف من جمع هذه العينات هو البحث العلمي فقط. وان جمع البيانات الخاصة بالمريض هي بيانات سريه لا يجوز الاضطلاع عليها إلا بعد موافقة المريض شخصيا".

كما نتعهد بسلامه المريض وخلو جميع المواد والمعدات المستخدمة من الأمراض المعدي هاو المضاعفات التي يمكن حدوثها ،كما أن جميع النتائج ستظل سريه .

(هذه الأبحاث تسهم في التعرف على مضاعفات بعض الأمراض وبالتالي تساعد في تجنب هذه المضاعفات مما يسهم في سرعه الاستشفاء)

ولكم الشكر،،،،،

اسم الباحث.. رقم الهاتف ....

اقر أنا .... بالموافقة على المشاركة في هذا البحث علما" بأنه تم اطلاعي على كل المعلومات المطلوبه.